REMARKS

The final Office Action mailed May 5, 2009, has been received and carefully considered. The Examiner's allowance of claim 24 is acknowledged with thanks.

From Public P.A.I.R., Applicants note that it appears that the Second Substitute Specification submitted on February 26, 2009, has not been entered into the record. Since the Examiner has objected to the Specification and required further changes to the Specification on page 6 of the Office Action, a Third Substitute Specification is attached, as well as a Marked-up copy of the Substitute Specification of record showing the changes made.

In this Amendment, claims 16-21 have been amended. To the best of the undersigned attorney's information and belief, these changes contain no new matter for the reasons given below.

Claims 16-27 are pending in the Application and are submitted to be in allowable condition. Claims 16, 24, and 25 are independent. Claim 24 has been allowed.

Claim Changes and Support

Independent claim 16 has been amended as the Examiner suggests to add *"and salts thereof"* so that the objection to claims 17-19, 22, and 23 is resolved.

Claims 17-21 have been amended to correspond to the changes made to claim 16.

I. The rejection of claims 16-13 and 25-27 under 35 U.S.C. §103(a) as unpatentably obvious over Baltzer or English, these in view of Xiong (2004), is respectfully traversed.

1. Applicants respectfully disagree with the Examiner's opinion (points 2-5 below) that the claims 16-23 and 25-27 are obvious to one of ordinary skill in this art in view of Baltzer or English, these in view of Xiong (2004), for the reasons given in the following (points 6 *et seq* below).

2. On page 2 of the Action, the Examiner considers that the Title of Baltzer teaches the concept of a mutual prodrug of β-lactam antibiotics and β-lactamase inhibitors, and that the generic wording in the paragraph bridging pages 1183-1184 sets out an advantage, namely, that *"both the antibiotic and the inhibitor are present simultaneously in appropriate balance at the site of the infection. This will usually not be the case when the two compounds are given as*

a combination ... because each drug in c a combination will have its own individual profile with respect to rate of absorption, distribution, duration of action." The Examiner thus contends, near the bottom of page 2 of the Action, that, "*This established that one of ordinary skill in the art would be well motivated to prepare the mutual prodrug rather than the combination of β-lactam antibiotic and β-lactamase inhibitor*".

3. Further, in the paragraph bridging pages 2 and 3 of the Action, the Examiner considers that "*English has a very similar teaching. Again, sulbactam is linked in the same way to a penicillin. ..... The 'Similar application to other drugs' would render such an approach obvious to any other drug which was already known to be synergistic with sulbactam."*

4. The Examiner further contends, in the first full paragraph on page 3 of the Action, that, *"The two examples of the primary reference, compounds 3 and 4, both employ sulbactam as the β-lactamase inhibitor. The β-lactam antibiotic in both cases is a penicillin. However, it would be obvious to use any "β-lactam antibiotic", as that is what the reference Baltzer teaches; again, see title and above cited paragraph. Likewise, English teaches 'other drugs' ".*

5. In the second full paragraph on page 3 of the Action, the Examiner considers that, *"In Xiong (2004), note Table 2, which shows strong synergism between sulbatam and Cephalothin, Cefuroxime, Cefpodoxime, Cefotaxime, Ceftazidime and Ceftriaxone. Note that cefuroxime is the second species in claim 16."*

6. All and all, therefore, it appears to Applicants that the Examiner has taken the view that because Penicillin has been indicated in the prior art, and because it is a sort of β-lactam antibiotic, all β-lactam antibiotics are deemed obvious because one of ordinary skill in the art would be motivated to achieve them by the teaching of the prior art.

7. Applicants respectfully believe that the Examiner's position is based on a mistaken concept.

8. First, there are very many products that may be considered to belong within β-lactam antibiotics. In support of this position, Applicants attach as **EXHIBIT - 1**, a copy of the pertinent text of the free encyclopedia Wikipedia website: http://en.wikipedia.org/wiki/Beta-lactam_antibiotic. The definition of β-lactam antibiotics given therein states, *"a broad class of antibiotics that include penicillin derivatives, cephalosporins, monobactams, carbopenems and β-lactamase inhibitors, i.e. any antibiotic agent that contains a β-lactam nucleus in its molecular structure"*. In addition, this text recites, *"They are the most widely-used group of antibiotics."*

9. Applicants wonder how the inventive step regarding a specific kind of the most widely-used group of antibiotics' be taken away by mere mention in the Title of Baltzer of, "mutual pro-drug of β-lactam antibiotics and β-lactamase inhibitors". Applicants additionally wonder how there can be any expectation of an advantage, as the Examiner's considers is found in English on page 346, *"There are several advantages inherent to carboxyl-terminated double-ester prodrugs for oral delivery of pharmaceutical agents. The carboxyl moiety imparts improved water solubility, especially as the pH rises, as in transit from the stomach to the small intestine. It also provides improved prospects for isolation of crystalline solids as free acids or as sodium salts, thus creating options to improve formulation of oral delivery forms. Another advantage is the formation of potentially innocuous organic diacids as by products after hydrolysis to the parent drug in vivo. Clinically, these advantages can be translated to drugs that are more efficacious, safe, and convenient to use. In summary, the acid-termination concept of ester-prodrug design has provided novel and effective delivery forms for the β-lactamase inhibitor sulbactam. Similar application to other drugs in orider to improve oral bio-availabilty, formulation, water solubility, and simultaneous byproducts formation in suggested".*

10. In particular, Applicants believe that it is not well founded for the Examiner to have concluded merely by the "Similar application to other drugs", as the Examiner stated, "would render such an approach obvious to any other drug which was already known to be synergistic with sulbactam".

11. To clarify what the chemical structure of Penicillin and Cephalosporin is, Applicants attach hereto **Exhibit - 2** and **Exhibit - 3** from Wikipedia (mentioned above), showing the structure of Penicillin and Cephalosporin, respectively. The common structure of both is the core structure, a Beta-lactam ring or penam, which is possessed by all β-lactam antibiotics, even through each of them has its own unique complete structure.

12. In Baltzer and English, the specific sort of β-lactam antibiotic, Cephalosporin, has not been mentioned at all. In particular, no preparation of a series of β-lactamase resistant cephalosporin ester compound and salts thereof, as well as their use for preparation of the antibiotics, has been disclosed or suggested. This same situation applies to Xiong (2004).

13. A "selection patent concept" might be applied herein. As Lord Wilberforce judged in the Case **Du Pont's (Witsiepe) Application [1982] F.S.R. 303, H:**

"The law regarding selection patents has been developed to deal with this problem. It has done so in the direction of recognizing two objectives, first to protect the original inventor, as regards the invention which he has made, but secondly, to encourage other researchers in the field to use their inventive powers so as to discover fresh advantages and to treat the discovery of such advantage as inherent in selected members of the group or class as a patentable invention. The modern statement of this part of the law as regards chemical patents is the judgment of Maugham J. in I.G. Farbenindustrie AG's Patents (1930) 47 R.P.C. 289, a case concerned with chemical combinations for the production of dyes. It has been approved and carried forward in cases concerned with the production of synthetic penicillins, where again the number of possible molecular variations is very large. The present position was compendiously stated by Lort Diplock: 'The patents at any rate to the extent that they claim the products para-hydroxy-penicillin and Amoxycillin respectively, are selection patents.

The inventive step in a selection patent lies in the discovery that one or more members of a previously known class of products possess some special advantage for a particular purpose, which could not be predicted before the discovery was made (in Re. I.G. Farbenindustrie AG's Patents (1930) 47 R.P.C. 283 per Maugham J. at 322-323). The quid pro quo for the monopoly granted to the invention is the public disclosure by him in his specification of the special advantages that the selected members of the class possess.' (Beecham Group Ltd v Bristol Laboratories International SA [1978] R.P.C. 521 at 579).

My own opinion contains observations to a similar effect – loc. cit., p.568."

Further, Lord Wilberforce stated in the judgment, "In the first place, in order to leave open a field for selection by a subsequent inventor, it does not matter whether the original field is described by formula or by enumeration. A skilled chemist could, in most cases, quite easily transform the one into the other and the rights of the subsequent inventor cannot depend upon the notation used. ...

Secondly, the size of the initial group or class is not in itself decisive as to a question of prior publication of an invention related to a selected member or members. A selection patent might be claimed for one or several out of a class of 10 million (cf. I.G. Farbenindustrie AG's Patents at 321) or for one out of two (cf. the selection of one of two epimers of a synthetic penicillin combination). The size of the class may be

relevant to a question of obviousness, and that question in turn may depend, in part, upon whether the later invention relates to the same field as that occupied by the prior invention, or to a different field. If an ordinary uninventive man would not be likely to look for the advantages he desires to produce in the area occupied by the prior invention, a decision to do so may well amount to the beginning of an inventive step. Here, to look for a product possessing special thermoplastic and elastomeric qualities in a 20-year-old patent concerned with producing dyeable fibres involves, prima facie, an inventive approach.

Thirdly, disclosing a prior invention does not amount to prior publication of a later invention if the former merely points the way which might lead to the latter. A much quoted and useful passage is that from the judgment of the Court of Appeal in General Tire & Rubber Co v. Firestone Tyre & Rubber Co [1972] R.P.C. 456 at 486. There, Sachs L.J. said: 'A signpost, however clear, upon the road to the patentee's invention will not suffice. The prior inventor must be clearly shown to have planted his flag at the precise destination before the patentee.'

Attractive metaphors may be dangerous for those in search of precision, but the passage illustrates the necessity that the alleged prior disclosure must clearly indicate that use of the relevant material (i.e. that ultimately selected) does result in a product having the advantages predicted for the class. The point is well put by the New Zealand Court of Appeal. Dealing with semi-synthetic penicillin, the court (per Cooke J.) said: 'If such a compound has not been made before, its properties often cannot be predicted with any confidence: and where that is the case we do not consider that the invention claimed can fairly or accurately be described as 'published', even if a skilled chemist would realize that to make the compound by routine means would be practicable. A making of the compound and a discovery of its properties is necessary before the 'invention' has occurred and can be published.'

It is the absence of the discovery of the special advantages, as well as the fact of non-making, that makes it possible for such persons to make an invention related to a member of the class....."

14. Applying the law as stated in the foregoing, it is no doubt that the present invention was not disclosed or published by the cited prior art, nor might be arrived at by motivated skilled artisans. The latter merely indicated a concept with title or an implication of a mutual prodrug is

expected to be prepared, as Baltzer did, or with a mere wording "Similar application to other drugs", as English did, which are in fact general comments from a person skilled in the art or a merely simple expectation of the persons in the field, but in the entire absence of detail information pertaining to a making of the compound and discovery of its properties with description in the disclosed papers. This may demonstrate, on the other hands, the age of the references (1980, 1989). That is, a problem wanted to be solved, or an expectation or a desire bearing in the mind of artisans in the field that may have a such kind drug has been taken a long time, however no such a kind drug has been really presented until the present invention.

15. Further, we refer to the case of **Bayer's (Baatz – Carbonless Copying Paper) Application [1982] O.J. EPO 206, Tech. Bd. App.** This application concerned a carbonless copying paper. The material on the surface of the paper which would act in place of carbon sheet was a dye contained in micro-capsules. An earlier published specification (German application, No.2,251,381) revealed that the walls of the capsules could be made of a particular class of polyisocyanates. The claimed invention was said to lie in the selection of a sub-group of this class. The Examining Division held the selection obvious. But on appeal, the applicants produced evidence of comparative tests showing, as the advantage of the selection, that the paper did not so rapidly lose its copying capacity.

Referring to this prior art, the Board of Appeal stated:

"The indisputable novel carbonless copying papers according to the application differ from these in that a more precisely presented oxadiazinetrione diisocyanate is employed as polyisocyanate. To this extent the problem could be seen as the mere preparation of another carbonless copying paper ... As shown in comparative experiments 20-22, the applicant had defined the problem, vis-a-vis the nearest prior art as not just preparing other copying papers but improved copying papers."

Referring to the prior art already mentioned, the Board stated:

"But from the point of view of the problem of preparing copying paper having improved storage stability, the prior art cited by the Examining Division did not give any indication for selection of the more precisely prescribed oxadiazinetrione diisocyanate according to the claim from the enormous number of possible polyisocyanates for the micro-encapsulation of dyestuff-intermediates."

Referring to another similar prior specification, the Board said:

"The document does not furnish any inducement to employ the oxadiazinetrione diisocyanates for the production of improved copying papers: since this special class of compounds is merely mentioned incidentally and not emphasized by an example. Mere mention of a group of substances amongst numerous other groups of substances permits at best a surmise of comparable suitability and effectiveness for the purpose in hand if these groups of substances are interchanged."

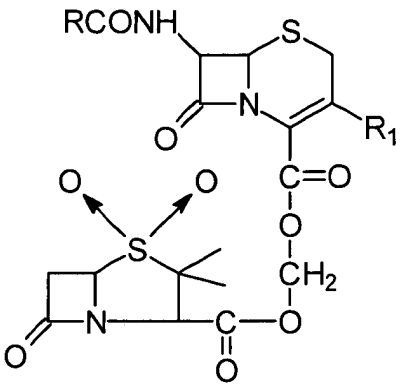
A further prior specification covered, inter alia, polyisocyanates within the current application for use as coatings for wood, metal and the like and for moulding and foam. The Board commented:

"Even with knowledge of this prior art the person skilled in the art could at the most expect that if he were to use these oxadiazinetrione diisocyanates to produce wall material for micro-capsules he would get only qualitatively and quantitatively similar results as in German unexamined application 2,251,381. Thus the person skilled in the art who-as in this case-had tried to improve the copying the copying papers specified in German unexamined application 2,251,381 would, on the basis of the prior art cited, not having arrived at the solution claimed in the application. The teaching of the present application, that the employment of the oxadiazinetrione diisocyanates as claimed leads to significantly improved copying papers must - independently of whether it is expressed in the form of the product claim 1 or the use claim 2 – be regarded as surprising, and hence involving an inventive step within the meaning of EPC Art. 56."

16. **Please note the content of EPC Art. 56 is similar or almost identical with the meaning of 35 USC§103.**

17. Applying the above case to the present invention, Applicants submit that the β-lactamase resistant cephalosporin ester compound and salts thereof according to the present application have indisputable novelty which differs, as being more precisely presented, from the combined disclosuresof Baltzer or English taken with Xiong (2004). Applicants' β-lactamase resistant cephalosporin ester compound is, characterized in that the structures of the compound are composed by connecting methyl ester residue of sulbactam halomethyl ester with carboxyl residue of semi-synthetic cephalosporin or salts thereof is employed together with what the inorganic salt can be as, as recited in Applicants' Specification pages 2-3, and supported by the whole content of the Application. To this extent the problem of the present

invention could be seen as the preparation of another compound or pharmaceutical salts thereof, characterized in that the compound is represented by the following formula (I):



which is recited in the original description on page 3 and the currently amended claim 16.

18. From the point of view of the problem addressed by the present invention, the prepared β-lactamase resistant cephalosporin ester compound and salts thereof have *improved* antibacterial effect, e.g., as presented in the Experimental results on page 21 and pages 23-24 respectively. In contrast, the combined disclosures of Baltzer or Englich taken with Xiong (2004) does not give any indication for selection of the more precisely prescribed β-lactamase resistant cephalosporin ester compound and salts thereof according to the currently amended claims from among the enormous number of possible β-lactamase inhibitors and β-lactam antibiotics for an alleged mutual pro-drugs, in either Baltzer or English ytaken with Xiong (2004).

19. Thus, Applicants respectfully submit that the Examiner's contention on page 4 of the Action, paragraph" B", 3rd – 5th sentances, that, *" Baltzer and English both made the mutual prodrug of β-lactam antibiotics and β-lactamase inhibitors, and both formed them in the exact same way applicants do, by esterifying both the β-lactam antibiotic and β-lactamase inhibitor to the same methylene group. The only reason that these references don't anticipate is that that neither used the exact permutation of β-lactam antibiotic and β-lactamase inhibitor. The solution of finding something better than a simple mixture of β-lactam antibiotic and β-lactamase inhibitor was already known.* is mistaken, not well founded, and should be withdrawn. Instead, the underlined part above should be represented more accurately *as "an expectation is already anticipated"*. Again, Applicants believe that an expectation or an anticipation, without selection

of the more precisely prescribed substances, cannot take away the novelty as well as the non-obviousness of the presently pending claims.

20. With regards to the Examiner's position on page 3 of the Action, the second full paragraph, that, *"In Xiong (2004), note Table 2, which shows strong synergism between sulbactam and Cephalothin, Cefuroxime, Cefpodoxime, Cefotaxime, Ceftazidime and Ceftriaxone. Note that cefuroxime is the second species in claim 16."*, Applicants respectfully submit that the Examiner made a conclusion based on a wrong concept as well. Table 2 merely show "*Results of susceptibility testing for transformants*", which, as stated by Xiong (2004) on page 266, under "4. Discussion", merely indicates "...the **possibility** of horizontal transfer of the resistance gene." Please note that therein is no indication in Xiong (2004) for selection of the more precisely prescribed β-lactamase resistant, nor any improved antibacterial effect having been given unlike Applicants' Experiment results on page 21 and pages 23-24 of the present Application provide.

21. In view of the foregoing points, Applicants respectfully submit that no *prima facie* case of obviousness has been made out by the combined disclosures of Baltzer or English in view of Xiong (2004) so that these grounds of rejection should be withdrawn.

22. With regards to the Examiner's rebuttal in paragraph "C" bridging pages 4 and 5 of the Action, that, "*It's not at all clear what specifically applicants refer to, but even if true, that would only be relevant to method of manufacture claims. If applicants are saying that the sulbactam esters cannot be made except for methods which applicants invented, then such an argument could overcome the rejection. However, applicants are not actually saying that, and it's unclear how applicants could make such an argument, given that e.g. English certain seems to say that they can make the sulbactam esters.*", Applicant wishes to clarify that independent claim 16 is a material claim, which should be supported or may only be conferred a patent right by the prescribed methods with which the materials are invented.

23. Applicants respectfully submit that the Examiner has misunderstood the references. That is, the methods disclosed in English are not a same, and do not even pertain to the precisely prescribed substances as recited in Applicants' claim 16, which is β-lactamase resistant cephalosporin ester compound and salts thereof.

24. With regards to the Examiner's rebuttal in paragraph "E", Applicants point out that it would be common knowledge to one of ordinary skill in this art that a possibility of an antibacterial drug activity to certain bacterial strain or strains is determined by certain

antibacterial drug(s), which directs to a topic of definition of an antibacterial, and falls in the range of pharmacology. Anti-bacterial antibiotics can be categorized based on their target specificity: "narrow-spectrum" antibiotics target particular types of bacteria, such as Gram-negative or Gram-positive bacteria, while broad-spectrum antibiotics affect a wide range of bacteria. Each of them has itself use purpose, and it is not relevant to validity of anti-bacterial antibiotics. Due to this common knowledge of the artisan, Applicants have not given a greater description in this regard, but suggest leaves this to the Examiner to ponder. For example, in Applicants' Experimental results listed under item 5 on page 21, a worse effect of YR-1 and YR-2 was obtained compared to the parent substance, e.g., Staphylococcus 26003 does not affect the validity of YR-1 and YR-2, provided for certain bacterial strains, YR-1 and YR-2 showed better effect, e.g., in Bacillus proteus 49085.

25. Similar arguments apply to the "Experimental result" under item 6, on page 23-24. The *improved* effects of anti-bacterial in certain bacterial strain(s) in conjunction with more convenient and easier administrating way make the compound significant in the light of citations.

26. Furthermore, in the case **Mobey Chemical's Application [1982] O.J. EPO 394, Tech. Bd. App.** The Applicants claimed a process for producing MBP (methylene bisphenyl isocyanate) which was liquid and stable in storage by heating the substance in the presence of catalyst (P.O.- phosopholine acid) to a temperature pf 1808 to 3008C and then quenching it to 1008C or less. This was said to be an improvement over the prior art in which quenching was not used and the catalyst has to be removed by the use of a poison which itself had undesirable consequences. Alternatively higher temperatures had to be used.

27. ***The Tech Bd's conclusion was***: *"In summary it is clear that, given the problem to be solved, neither the methods of the prior art individually, nor their respective combination with the generally available specialist knowledge, would make the solution according to the invention with the advantageous effects achieved foreseeable."* This Applicants consider to be very apropos to Applicants' Application and claims.

28. With reference to all of the above cases, and bearing the correct concept in assessment of the non-obvious and/or inventive step in minds, Applicants conclude as follows:

The precisely prescribed β-lactamase resistant cephalosporin ester compound, which characterized in that the structures of the compound are composed by connecting methyl ester residue of sulbactam halomethyl ester with carboxyl residue of semi-synthetic cephalosporin or

salts thereof is employed together with the inorganic salts precisely recited, in conjunction with the teaching manufacture method of the compound, and the experimental results demonstrating the improved antibacterial effects to certain bacterial strains, represent a valuable antibacterial drug, which is non-obvious to a person skilled in the art in the light of citations, but with inventive step.

29. In view of the foregoing points, Applicants respectfully submit that no *prima facie* case of obviousness has been made out by the combined disclosures of Baltzer or English in view of Xiong (2004) so that these grounds of rejection should be withdrawn.

II. The objection to claims 17-19, 22, and 23 as reciting salts, but salts are not provided in claim 16 is believed resolved.

The Examiner's suggestion to amend independent claim 16 to recite "and salts thereof" has been adopted and a period has been added at the end of claim 16.

III. The requirement that an amended Specification be filed to remove Chinese characters from all of the tables and to rename "acetdimethylamide" where found is believed satisfied by the Third Substitute Specification submitted herewith.

From Public P.A.I.R., Applicants note that it appears that the Second Substitute Specification submitted on February 26, 2009, has not been entered into the record. Since the Examiner has objected to the Specification and required further changes to the Specification on page 6 of the Office Action, a Third Substitute Specification is attached, as well as a Marked-up copy of the Substitute Specification of record showing the changes made.

With reference to the tables e.g., the top of page 9, the Examiner appears to be taking the position that " υ " in "chemical shift (υ)" is a Chinese character. This is submitted to not be the case. Rather, "(υ) " is the Greek letter "Y", upsilon, shown in lower case as " υ ". The font used in the Application as-filed is Times New Roman where the Greek letter "Y", upsilon, is submitted to be properly shown in lower case as " υ ".

However, since "chemical shift (υ)" appears only at the top of six tables and not in the text or claims *per se*, Applicants propose to delete " υ " from this expression as not necessary and as reducing possible confusion.

Further, as seen in the attached Marked-up Substitute Specification, all instances of "acetdimethylamide" have been corrected to "dimethyl acetamide".

In view of these changes, Applicants believe that the objection to the Specification has been resolved so that this objection should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that claims 16-27 and the Application are in condition for allowance. Reconsideration and passage of this case to issue are therefore requested.

Should the Examiner consider that a conference would help to expedite the prosecution of this Application, the Examiner is invited to contact the undersigned to arrange for such an interview.

Other than the $405.00 fee accompanying the filing of A Request For Continued Examination, no other fee is believed due. This fee is submitted herewith in the attached credit card form PTO-2038. Should the remittance be accidentally missing or insufficient, the Commissioner is hereby authorized to charge the fee to our Deposit Account No. 18-0002 and is requested to advise us accordingly.

Respectfully submitted,

August 5, 2009
Date

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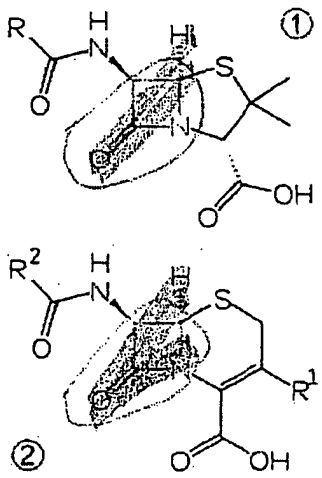
SMR/AJW:ac

EXHIBIT 1

Beta-lactam antibiotic

From Wikipedia, the free encyclopedia

β-lactam antibiotics are a broad class of antibiotics that include penicillin derivatives, cephalosporins, monobactams, carbapenems, and β-lactamase inhibitors,[1] that is, any antibiotic agent that contains a β-lactam nucleus in its molecular structure. They are the most widely-used group of antibiotics.



Core structure of penicillins (top) and cephalosporins (bottom). Beta-lactam ring in red.

Contents

Clinical use

β-lactam antibiotics are indicated for the prophylaxis and treatment of bacterial infections caused by susceptible organisms. At first, β-lactam antibiotics were mainly active only against Gram-positive bacteria, yet the recent development of broad-spectrum β-lactam antibiotics active against various Gram-negative organisms has increased their usefulness.

Mode of action

β-Lactam antibiotics are bactericidal, and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity, especially in Gram-positive organisms. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases known as penicillin-binding proteins (PBPs).

β-Lactam antibiotics not only block the division of bacteria, including cyanobacteria, but also the

division of cyanelles, the photosynthetic organelles of the Glaucophytes, and the division of chloroplasts of bryophytes. In contrast, they have no effect on the plastids of the highly developed vascular plants. This is supporting the endosymbiotic theory and indicates an evolution of plastid division in land plants [2].

β-lactam antibiotics are analogues of D-alanyl-D-alanine - the terminal amino acid residues on the precursor NAM/NAG-peptide subunits of the nascent peptidoglycan layer. The structural similarity between β-lactam antibiotics and D-alanyl-D-alanine facilitates their binding to the active site of penicillin-binding proteins (PBPs). The β-lactam nucleus of the molecule irreversibly binds to (acylates) the Ser_{403} residue of the PBP active site. This irreversible inhibition of the PBPs prevents the final crosslinking (transpeptidation) of the nascent peptidoglycan layer, disrupting cell wall synthesis.

Under normal circumstances peptidoglycan precursors signal a reorganisation of the bacterial cell wall and, as a consequence, trigger the activation of autolytic cell wall hydrolases. Inhibition of cross-linkage by β-lactams causes a build-up of peptidoglycan precursors, which triggers the digestion of existing peptidoglycan by autolytic hydrolases without the production of new peptidoglycan. As a result, the bactericidal action of β-lactam antibiotics is further enhanced.

Modes of resistance

By definition, all β-lactam antibiotics have a β-lactam ring in their structure. The effectiveness of these antibiotics relies on their ability to reach the PBP intact and their ability to bind to the PBP. Hence, there are 2 main modes of bacterial resistance to β-lactams, as discussed below.

The first mode of β-lactam resistance is due to enzymatic hydrolysis of the β-lactam ring. If the bacteria produces the enzymes β-lactamase or penicillinase, these enzymes will break open the β-lactam ring of the antibiotic, rendering the antibiotic ineffective. The genes encoding these enzymes may be inherently present on the bacterial chromosome or may be acquired via plasmid transfer, and β-lactamase gene expression may be induced by exposure to beta-lactams. The production of a β-lactamase by a bacterium does not necessarily rule out all treatment options with β-lactam antibiotics. In some instances, β-lactam antibiotics may be co-administered with a β-lactamase inhibitor. However, in all cases where infection with β-lactamase-producing bacteria is suspected, the choice of a suitable β-lactam antibiotic should be carefully considered prior to treatment. In particular, choosing appropriate β-lactam antibiotic therapy is of utmost importance against organisms with inducible β-lactamase expression. If β-lactamase production is inducible, then failure to use the most appropriate β-lactam antibiotic therapy at the onset of treatment will result in induction of β-lactamase production, thereby making further efforts with other β-lactam antibiotics more difficult.

The second mode of β-lactam resistance is due to possession of altered penicillin-binding proteins. β-lactams cannot bind as effectively to these altered PBPs, and, as a result, the β-lactams are less effective at disrupting cell wall synthesis. Notable examples of this mode of resistance include methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae*. Altered PBPs do not necessarily rule out all treatment options with β-lactam antibiotics.

Common β-lactam antibiotics

Penicillins

Semisynthetic penicillins are prepared starting from the penicillin nucleus 6-APA.

Narrow-spectrum

- Beta-lactamase sensitive
  - benzathine penicillin
  - benzylpenicillin (penicillin G)
  - phenoxymethylpenicillin (penicillin V)
  - procaine penicillin
- Penicillinase-resistant penicillins
  - methicillin
  - oxacillin[3]
  - nafcillin
  - cloxacillin
  - dicloxacillin
  - flucloxacillin
- β-lactamase-resistant penicillins
  - temocillin

Moderate-spectrum

- amoxycillin
- ampicillin

Broad-spectrum

- co-amoxiclav (amoxicillin+clavulanic acid)

Extended-spectrum

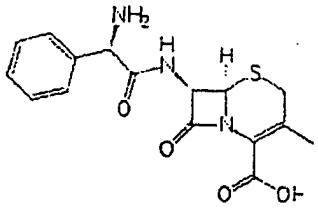
- azlocillin
- carbenicillin
- ticarcillin
- mezlocillin
- piperacillin

Cephalosporins

First generation

Moderate spectrum.

- cephalexin
- cephalothin
- cefazolin



Skeletal formula of cefalexi
a first-generation
cephalosporin

Second generation

Moderate spectrum with anti-*Haemophilus* activity.

- cefaclor
- cefuroxime
- cefamandole

Second generation cephamycins

Moderate spectrum with anti-anaerobic activity.

- cefotetan
- cefoxitin

Third generation

Broad spectrum.

- ceftriaxone
- cefotaxime
- cefpodoxime

Broad spectrum with anti-*Pseudomonas* activity.

- ceftazidime

Fourth generation

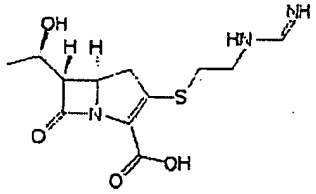
Broad spectrum with enhanced activity against Gram positive bacteria and beta-lactamase stability.

- cefepime
- cefpirome

Carbapenems

Broadest spectrum of beta-lactam antibiotics.

- imipenem (with cilastatin)
- meropenem
- ertapenem
- faropenem
- doripenem



Skeletal formula of imipenem

Monobactams

Unlike other beta-lactams, the monobactam contains a nucleus with no fused ring attached. Thus, there is less probability of cross-sensitivity reactions.

- aztreonam (Azactam)

Beta-lactamase inhibitors

Although they exhibit negligible antimicrobial activity, they contain the beta-lactam ring. Their sole purpose is to prevent the inactivation of beta-lactam antibiotics by binding the beta-lactamases, and as such, they are co-administered with beta-lactam antibiotics.

- clavulanic acid
- tazobactam
- sulbactam

Adverse effects

Adverse drug reactions

Common adverse drug reactions (ADRs) for the β-lactam antibiotics include diarrhea, nausea, rash, urticaria, superinfection (including candidiasis).[4]

Infrequent ADRs include fever, vomiting, erythema, dermatitis, angioedema, pseudomembranous colitis.[4]

Pain and inflammation at the injection site is also common for parenterally-administered β-lactam antibiotics.

Allergy/hypersensitivity

Immunologically-mediated adverse reactions to any β-lactam antibiotic may occur in up to 10% of patients receiving that agent (a small fraction of which are truly IgE-mediated allergic reactions, see amoxicillin rash). Anaphylaxis will occur in approximately 0.01% of patients.[4][5] There is perhaps a 5%-10% cross-sensitivity between penicillin-derivatives, cephalosporins, and carbapenems; but this figure has been challenged by various investigators.

Nevertheless, the risk of cross-reactivity is sufficient to warrant the contraindication of all β-lactam antibiotics in patients with a history of severe allergic reactions (urticaria, anaphylaxis, interstitial nephritis) to any β-lactam antibiotic.

Jarish Herxheimer reaction: Febrile reaction after first injection of penicillin in spirochetal infection. Eg; Syphilis

References

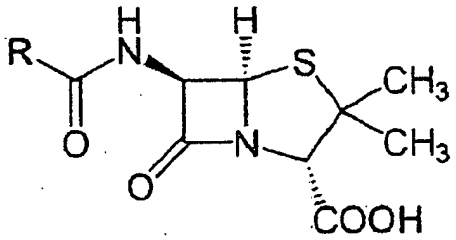
1. ^ Holten KB, Onusko EM (August 2000). "Appropriate prescribing of oral beta-lactam antibiotics". *American family physician* **62** (3): 611–20. PMID 10950216. http://www.aafp.org/afp/20000801/611.html.
2. ^ Britta Kasten und Ralf Reski (1997): β-lactam antibiotics inhibit chloroplast division in a moss (Physcomitrella patens) but not in tomato (Lycopersicon esculentum). Journal of Plant Physiology 150, 137-140 [1]
3. ^ "Mayo Clinic Proceedings". http://www.mayoclinicproceedings.com/inside.asp?AID=2547. Retrieved on 2008-12-26.
4. ^ [a] [b] [c] Rossi S (Ed.) (2004). *Australian Medicines Handbook 2004*. Adelaide: Australian Medicines Handbook. ISBN 0-9578521-4-2.
5. ^ Pichichero, Michael E. "A Review of Evidence Supporting the American Academy of Pediatrics Recommendation for Prescribing Cephalosporin Antibiotics for Penicillin-Allergic Patients." *Pediatrics*. 2005, 115 1048-1057. PMID 15805383

EXHIBIT 2

Penicillin

From Wikipedia, the free encyclopedia

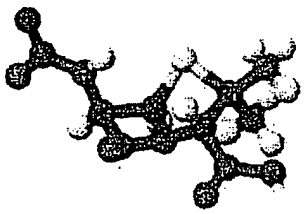
Penicillin (sometimes abbreviated **PCN** or **pen**) is a group of antibiotics derived from *Penicillium* fungi.[1] Penicillin antibiotics are historically significant because they were the first drugs that were effective against many previously serious diseases such as syphilis and Staphylococcus infections. Penicillins are still widely used today, though many types of bacteria are now resistant. All penicillins are Beta-lactam antibiotics and are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms.



Penicillin core structure. "R" is variable group.

The term "penicillin" can also refer to the *mixture* of substances that are naturally produced.[2]

The term "penam" is used to describe the core skeleton of a member of a penicillin antibiotic. This skeleton has the molecular formula R-$C_9H_{11}N_2O_4S$, where R is a variable side chain.



Penicillin core structure, in 3D. Purple is variable group.

Contents

History

Discovery

The discovery of penicillin is attributed to Scottish scientist and Nobel laureate Alexander Fleming in 1928. He showed that if *Penicillium notatum* was grown in the appropriate substrate, it would exude a substance with antibiotic properties, which he dubbed penicillin. This serendipitous observation began the modern era of antibiotic discovery. The development of penicillin for use as a medicine is attributed to the Australian Nobel laureate Howard Walter Florey together with the German Nobel laureate Ernst Chain and the English biochemist Norman Heatley.

However, several others reported the bacteriostatic effects of *Penicillium* earlier than Fleming. T first published reference appears in the publication of the Royal Society in 1875, by John Tyndall.[3]

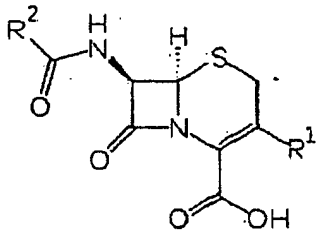
EXHIBIT 3

Cephalosporin

From Wikipedia, the free encyclopedia

The **cephalosporins** (IPA: /ˌsɛfələʊˈspɔərɪn/) are a class of β-lactam antibiotics originally derived from *Acremonium*, which was previously known as "Cephalosporium".[1]

Together with cephamycins they constitute a subgroup of β-lactam antibiotics called cephems.



Core structure of the cephalosporins

Contents

History

Cephalosporin compounds were first isolated from cultures of *Cephalosporium acremonium* from a sewer in Sardinia in 1948 by Italian scientist Giuseppe Brotzu [2]. He noticed that these cultures produced substances that were effective against *Salmonella typhi*, the cause of typhoid fever, which had beta-lactamase. Researchers at the Sir William Dunn School of Pathology at the University of Oxford isolated cephalosporin C. The cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA), was derived from cephalosporin C and proved to be analogous to the penicillin nucleus 6-aminopenicillanic acid, but it was not sufficiently potent for clinical use. Modification of the 7-ACA side-chains resulted in the development of useful antibiotic agents, and the first agent cephalothin (cefalotin) was launched by Eli Lilly in 1964.

Mode of action

Cephalosporins are bactericidal and have the same mode of action as other beta-lactam antibiotics (such as penicillins) but are less susceptible to penicillinases. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases known as penicillin binding proteins (PBPs). PBPs bind to the D-Ala-D-Ala at the end of muropeptides (peptidoglycan precursors) to crosslink the peptidoglycan. Beta-lactam

MARKED-UP COPY of Substitute Specification

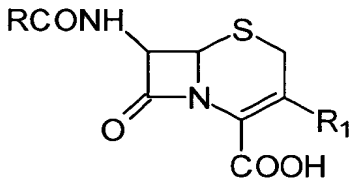
β-lactamase Resistant Cephalosporin Ester Compounds and Salts of Thereof

Field of the Invention

This invention relates to a series of β-lactamase resistant cephalosporin ester compounds and salts of thereof, as well as their use for preparation of the antibiotics.

Background of the Invention

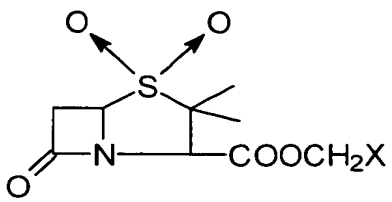
The compounds possessing the following formula (II) are all known semi-synthetic cephalosporin,



(II)

such as: cefetamet (CAS registration number 65052-63-3); cefuroxime (CAS registration number 55268-75-2); cefradine (CAS registration number 38821-53-3); cefalexin (CAS registration number 15686-71-2); cefaclor (CAS registration number 53994-73-3); and cefadroxil (registration number 50370-12-2). Among them, pivaloyloxymethyl ester of cefetamet (cefetamet pivoxil, CAS registration number 65243-33-6) and 1-(acetoxyl) ethyl ester of cefuroxime (cefuroxime axetil, CAS registration number 64544-07-6) along with another above-mentioned four kinds of cephalosporin are oral antibiotics which have been used in clinic.

The compound possessing the following formula (III) is:



(III)

Sulbactam (CAS registration number 68373-14-8) halogen methyl ester, which belongs to β-lactamase inhibitor, with strong irreversible inhibition to β-lactamase released by staphylococcus aureus and many other Gram negative bacteria. It manifests extremely strong inhibition to type II, III, IV, V β-lactamase at a concentration of 2μg/ml. If used with penicillin

and cephalosporin antibiotics, it can generate synergetic effects; currently, mixed injections of ampicillin, cefoperazone, cefotaxim, ceftriaxone and sulbactam sodium salt have been used in clinic, which can prevent these antibiotics from losing antibacterial activities due to being hydrolyzed by β-lactamase, reducing minimum inhibitory concentration of these antibiotics to certain drug resistant bacteria resulting from lactamase production.

It is well known that intravenous administration is time-consuming, and has the potential threats of blood-borne infectious disease such as hepatitis B, C, AIDS etc. For those mild, moderate inflammation patients or sequential therapy of patients after intravenous anti-inflammation therapy, it is usually sufficient of oral administration, which is not only convenient and safe, but also can save a lot of manpower, material resources and wealth. However, drug resistance is quite common among oral β-lactam antibiotics to lactamase-producing bacteria, thus resulting in poor therapeutic reactions. Therefore, preparation of oral β-lactamase resistant antibiotics is actually a focus topic in the field of antibiotics manufacture.

At present, bis-esters sultamicillin (CAS registration number 76497-13-7), which is synthesized chemically by the compounds (III) and ampicillin, is an oral antimicrobial being widely used in clinic; it can be hydrolyzed to ampicillin and sulbactam by esterase of intestine walls, thus exerting the same therapeutic effects as the mixed injection of sulbactam and ampicillin. However, there is yet no compound which can chemically synthesize the compounds (III) and cephalosporin and further prepare oral β-lactamase resistant antibiotics.

Summary of the Invention

The purpose of the present invention is to resolve the above topic, and to provide a β-lactamase resistant cephalosporin ester compound and salts of thereof.

The purpose of the present invention is accomplished by the following technical solution:

A β-lactamase resistant cephalosporin ester compound, the characterized in that the structures of the compound are composed by connecting methyl ester residue of sulbactam halomethyl ester with carboxyl residue of semi-synthetic cephalosporin or salts of thereof.

Wherein, salt of the semi-synthetic cephalosporin is inorganic salt or organic alkali salt.

The inorganic salt can be sodium salt, potassium salt, magnesium salt or calcium salt; the organic alkali salt can be triethylamine salt, tributylamine salt, 1.8-diazacyclo[5,4,0] undecane-7-ene salt, dicyclohexyl amine salt or tetrabutylammonium salt.

The semi-synthetic cephalosporin is slected from the group consisting of cefetamet, cefuroxime, cefradine, cefalexin, cefaclor or cefadroxil.

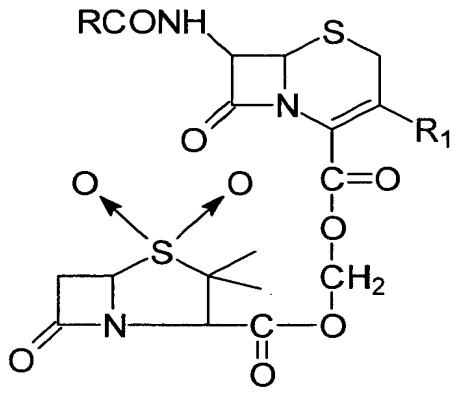
However, this sulbactam halomethyl ester can be sulbactam bromomethyl ester or sulbactam iodomethyl ester.

This invention also provides pharmaceutical salts of the above compound.

Wherein, this pharmaceutical salt is inorganic salt or organic acid salt.

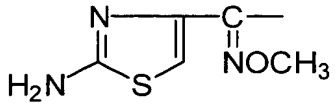
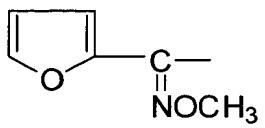
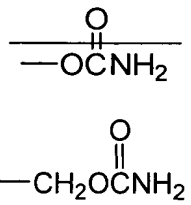
This inorganic salt or organic acid salt can be hydrochloride, sulphate, *p*-toluenesulfonate, tartrate, maleate and lactate.

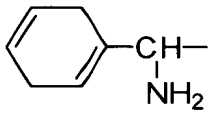
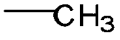
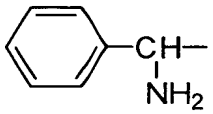
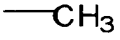
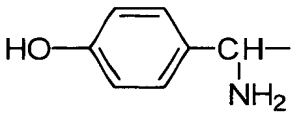
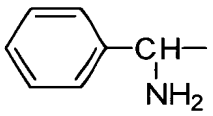
The compound or pharmaceutical salts thereof according to the invention, characterized in that the compound is represented by the following formula (I):



(I)

Wherein, the detailed meanings of R and R_1 are shown in the following table:

Compound (I) code	Compound (II)			
	Serial number	Common name	R	R_1
YR−1	II −1	cefetamet		
YR−2	II −2	cefuroxime		

YR—3	II —3	cefradine		
YR—4	II —4	cefalexin		
YR—5	II —5	cefadroxil		
YR—6	II —6	cefaclor		

Respective chemical names of this series of compounds (I) are listed as follows:

YR-1: 5-thia-1-aza-bicyclo [4,2,0] octane-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl) (methoxy imine) acetyl] amino]-3-methyl-8-oxo-, [[3,3-dimethyl-4,4-dioxy-7-oxo-4-thia-1-aza -bicyclo [3,2,0] heptane-2-group] carbonyloxy] oxy] methyl ester and salt thereof.

YR-2: 5-thia-1-aza-bicyclo [4,2,0] octane-2-ene-2-carboxylic acid, 7-[[(2-furane (methoxy imine) acetyl) amino]-3-[[(amino carbonyloxy) oxy] methyl]-8-oxo-, [[3,3-dimethyl-4,4-dioxy -7-oxo-4-thia-1-aza-bicyclo[3,2,0] heptane-2-group] carbonyloxy] oxy] methyl ester and salt thereof.

YR-3: 5-thia-1-aza-bicyclo [4,2,0] octane-2-ene-2-carboxylic acid, 7-[[amino-1,4-cyclohexadiene-1-group-acetyl] amino], 3-methyl-8-oxo-, [[3,3-dimethyl-4,4-dioxy-7-oxo-4-thia-1-aza-bicyclo [3,2,0] heptane-2-group] carbonyloxy] oxy] methyl ester and salt thereof.

YR-4: 5-thia-1-aza-bicyclo [4,2,0] octane-2-ene-2-carboxylic acid, 7-[[amino phenylacetyl] amino], 3-methyl-8-oxo-, [[3,3-dimethyl-4,4-dioxy-7-oxo-4-thia-1-aza-bicyclo [3,2,0] heptane -2-group] carbonyloxy] oxy] methyl ester and salt thereof.

YR-5: 5-thia-1-aza-bicyclo [4,2,0] octane-2-ene-2-carboxylic acid, 7-[[amino (4-

hydroxyphenyl)—acetyl] amino]-8-oxo-, [[3,3-dimethyl-4,4-dioxy-7-oxo-4-thia-1-aza-bicyclo [3,2,0] heptane-2-group] carbonyloxy] oxy] methyl ester and salt thereof.

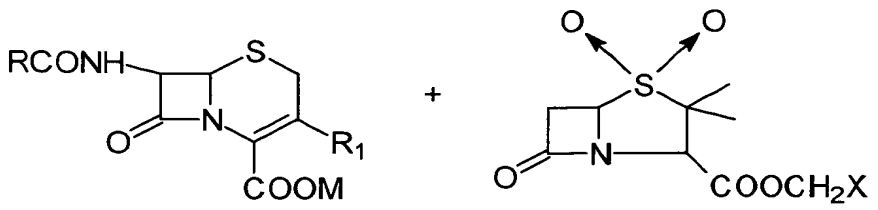
YR-6: 5-thia-1-aza-bicyclo [4,2,0] octane-2-ene-2-carboxylic acid, 7-[[amino phenylacetyl] amino], 3-cl-8-oxo-, [[3,3-dimethyl-4,4-dioxy-7-oxo-4-thia-1-aza-bicyclo [3,2,0] heptane-2-group] carbonyloxy] oxy] methyl ester and salt thereof.

The compounds and salts thereof according to the invention have the same intravital metabolic characteristic as that of sultamicillin; they can be hydrolyzed to cephalosporin and sulbactam by esterase of intestine walls after being administered orally, and the intravital synergistic effect can compensate the disadvantage of these cephalosporins being hydrolyzed by β-lactamase which is released by certain bacteria, thus reducing minimum inhibitory concentration to those certain drug resistant bacteria resulting from lactamase production to the sensitive range.

They can be synthesized by the compounds (II) and compound (III) through esterification reaction. The compounds (II) are cephalosporin antibiotics widely used in clinic, while the compound (III) can be synthesized according to the methods in 1984 <USP4,444,686> (Vytautas J.Jasys etc) and < pharmaceutical industry, 1985, 16 (8), 346-9> (Jiang Naicai etc).

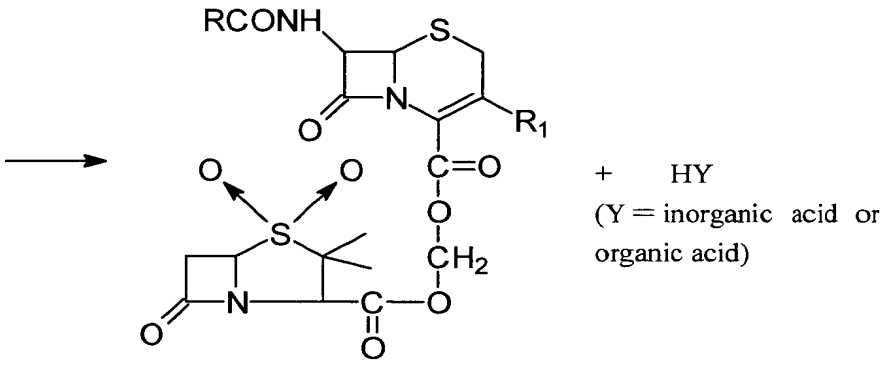
The compounds according to the invention can be synthesized by two distinct methods:

Method 1 (applicable to the synthesis of YR1-6)



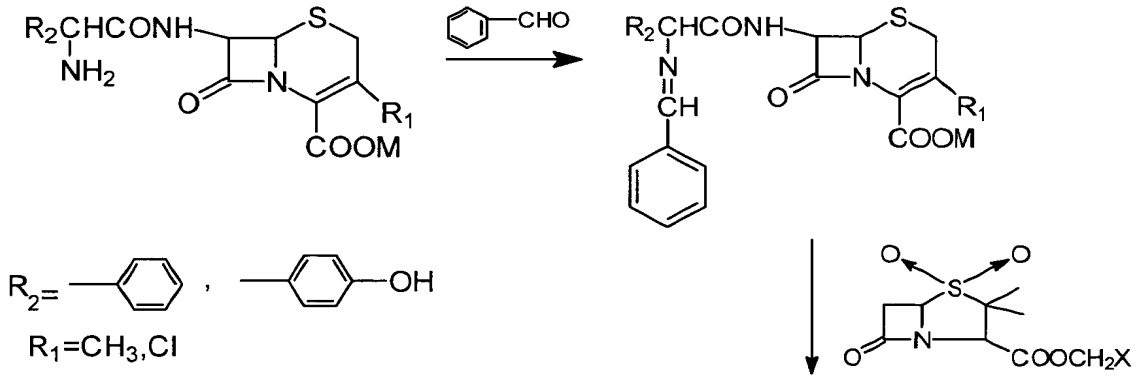
(M refers to metal or organic alkali

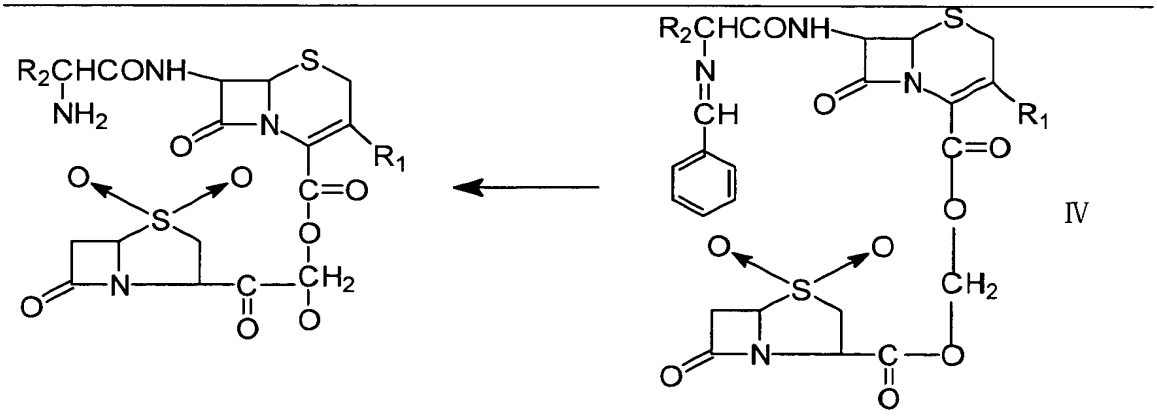
(X=I or Br)



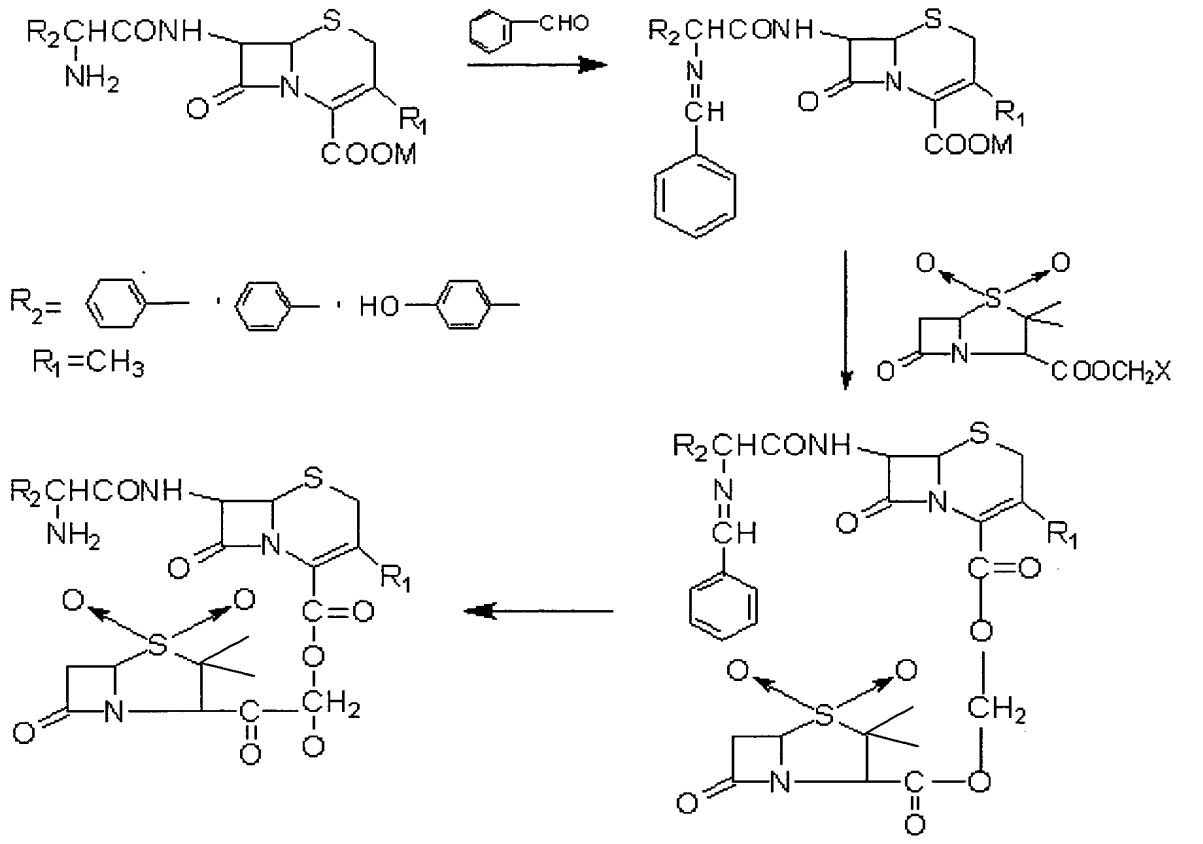
+ HY
(Y = inorganic acid or organic acid)

Method 2 (applicable to the synthesis of YR3-6)





IV



Method 1:

Salt of the compounds (II) such as sodium salt, potassium salt, magnesium salt, calcium salt, triethylamine salt, tributylamine salt, 1.8-diazacyclo [5,4,0] undecane-7-ene (DBU) salt, dicyclohexyl amine salt and tetrabutylammonium salt should be utilized when synthesizing the compounds (I) through method 1. The following examples introduce sodium salt, potassium salt, tributylamine salt and DBU salt of the compounds (II).

When synthesizing the compounds (I), the molar ratio of the compounds (II) and (III) can be from 1:0.9 to 1:1.5, and especially from 1:0.98 to 1:1. The reaction between the compounds (II) and (III) can occur at -15℃ to 30℃, and the reaction time generally varies from 30 minutes to 15 hours; adding 18 crownether-6, 16 crownether-4, 12 crownether-2, tetrabutyl ammonium hydrogen sulfate, tetrabutyl ammonium bromide during the process can promote the reaction.

Reaction solvent can be selected from the following substances: alkylogen such as dichloromethane, chloroform, dichloroethane etc; ketone such as acetone, cyclobutanone, cyclohexanone, methyl isobutyl ketone etc; polar aprotic solvent such as

~~acetdimethylamide~~dimethyl acetamide, dimethylformamide, dimethyl sulfoxide etc. The following examples introduce the reaction method using ~~acetdimethylamide~~dimethyl acetamide and dimethylformamide as solvent. The compounds and salts thereof according to the invention can be used to prepare oral antimicrobials, and the compounds according to the invention can be used to prepare a lot of inorganic salts and organic acid salts, such as hydrochloride, sulphate, *p*-toluenesulfonate, tartrate, maleate and lactate. The following examples introduce the preparation methods of *p*-toluenesulfonate and hydrochloride of the compounds according to the invention.

Method 2:

Method 2 is applicable to synthesize YR3-6, characterized in that the compounds (II) will react with benzaldehyde in the polar aprotic solvent such as dimethylformamide, ~~acetdimethylamide~~dimethyl acetamide or in the low-grade alcohol such as methanol, alcohol, protecting α-amino on the lateral chain and forming Shiff bases, then synthesize intermediate compounds (IV) through method 1, and finally, react with Grimard reagent to remove protecting group to produce the compounds I (YR3-6) and salts thereof.

Detailed Description of the Invention

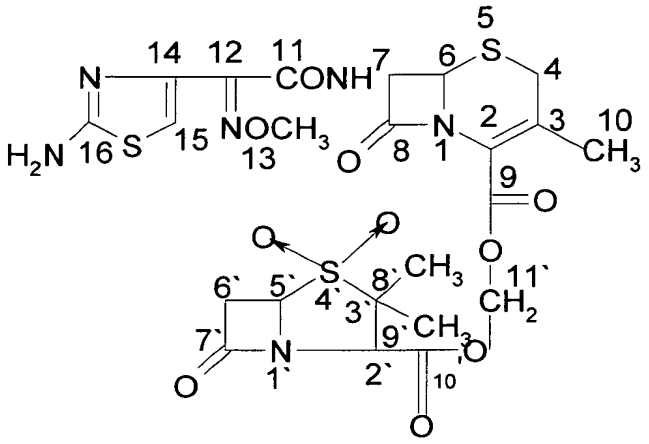
Example 1:

The potassium salt of compound (II-1) 11.0g (0.025mol) was suspended in 100ml of ~~acetdimethy-lamide~~ dimethyl acetamide, stirred evenly, added 0.5g of 18 crownether-6 to fully dissove, then cooled the solution to 0℃, added 9.4g (0.025mol) of the compound (III) (X=I), stirred for 30 minutes at 0℃, and controlled the reaction using thin-layer chromatography*. When the material spot disappeared, added 200ml of acetic ether and 200ml of water into the reaction solution, stirred thoroughly and delaminated, separated out water layer, extracted using 200ml of acetic ether, sequentially washed combined acetic ether layer with the mixture of 150ml water plus 5ml $NaHCO_3$ saturated aqueous solution and NaCl saturated aqueous solution, then decolored and dehydrated with activated carbon and magnesium sulfate anhydrous. Added 200ml of isopropanol into oily substances acquired after decompressing and evaporating acetic ether, stirred at room temperature for 1 hour, white precipitate was separated out, then filtered and washed the filter cake with small quantity of isopropanol, dryed at room temperature in vacuo, got 12.9g of white compound YR-1, 80% yield. High Pressure Liquid Chromatography showed that the purity was 98.5%.

*Silica gel plate HSGF254, developing agent isopropanol: ethyl acetate (2:1)

Compound (YR-1) Rf=0.8

The compound (I) was confirmed by IR and ^1H NMR



IR (KBr disc)

absorption peak (cm^{-1})	intensity	group
3454.53	Broad s	$-NH_2$
1784.53	Broad s	β-lactam
1734.4	Broad s	-COOR
1677.3	s	-CONH-
1623.31	s	-C=C-
1536.83	s	-C=N-
1320.76, 1120.38	s	-C-O-C-

^1H NMR(DMSO d_6)

chemical shift (~~υ~~)	genre
9.6004(d,1H, J=8.4 H_z)	-CONH
7.2335(broad s,2H)	$-NH_2$
6.7512(s,1H)	C_{15}—H
5.9545(Abq , 2H, J=6H_z)	$C_{11'}$—H
5.7445(dd, 1H, J=5H_z, 8H_z)	C_7—H
5.1903(m, 1H)	$C_{5'}$—H
5.1518(d,1H,J=5 H_z)	C_6—H
4.5297(s,1H)	$C_{2'}$—H

3.8352(s,3H)	C_{13}—H
3.6755(m,2H)	$C_{6'}$—H
3.6238,3.4619(ABq,2H,J=18.5H_z)	C_4—H
2.1007(s,3H)	C_{10}—H
1.4820(s,3H)	$C_{8'}$~~或~~ or $C_{9'}$—H
1.3765(s,3H)	$C_{8'}$~~或~~ or $C_{9'}$—H

Example 2:

Dissolved 4.6g (0.03mol) of DBU in 200ml of dimethylformamide, stirred and cooled to 0℃, added 13.1g (0.03 mol) of the compound (II-1) and 11.2g (0.03mol) of the compound (III) (X=I), reacted at below 0℃ for 30 minutes, trailed the reaction by thin-layer chromatography until the material spot disappeared. After the reaction finished, handled the reaction solution with the same method mentioned in example 1 and got 15.4g of the compound YR-1, 80% yield. High Pressure Liquid Chromatography showed that the purity was 98.2%. The analytic results of IR and ^{1}H NMR of the product was identical with those of example1.

Example 3:

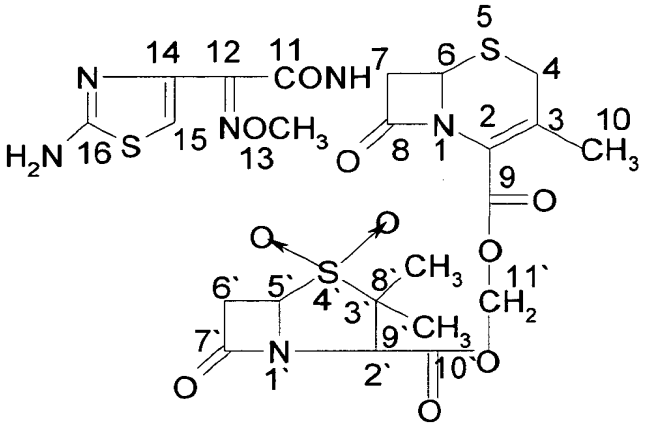
The potassium salt of compound (II-1) 11.0g (0.025mol) was suspended in 150ml of ~~acetdimethylamide~~dimethyl acetamide, stirred and controlled at 20℃~25℃, added 2.1g (0.006mol) of tetrabutyl ammonium hydrogen sulfate and 9.4g (0.025mol) of the compound (III) (X=I), reacted at the same temperature for 4~6hours, and trailed the reaction by thin-layer chromatography until the material spot disappeared. After the reaction finished, handled the reaction solution with the same method mentioned in example 1 and got 13.7g of the compound YR-1, 85% yield. High Pressure Liquid Chromatography showed that the purity was 98.7%. The analytic results of IR and ^{1}H NMR of the product were identical with those of example1.

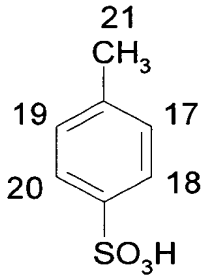
Example 4:

Stirred 6.45g (0.01mol) of the compound (YR-1) (got from example 2) at room temperature, dissolved in 65ml of acetic ether, added 2.1g (0.012mol) of *p*-toluenesulfonic acid and stirred until solids were separate out, continued stirring for another 3 hours, filtrated, washed the solids with small quantity of acetic ether, dried in vacuo and got 7.2g white *p*-toluenesulfonate of the compound (YR-1), 88% yield. High Pressure Liquid Chromatography showed that the

purity was 98.5%.

P-toluenesulfonate of the compound (YR-1) was confirmed by IR and ^{1}H NMR





IR (KBr disc)

absorption peak cm^{-1}	intensity	group
3456	Broad s	$-NH_2$
1784.96	Broad s	β-lactam
1675.89	s	1675.89
1638.61	s	-C=C-
1541.32	s	-C=N-
1321.64, 1121.9	s	-C-O-C-

^{1}H NMR (DMSO d_6)

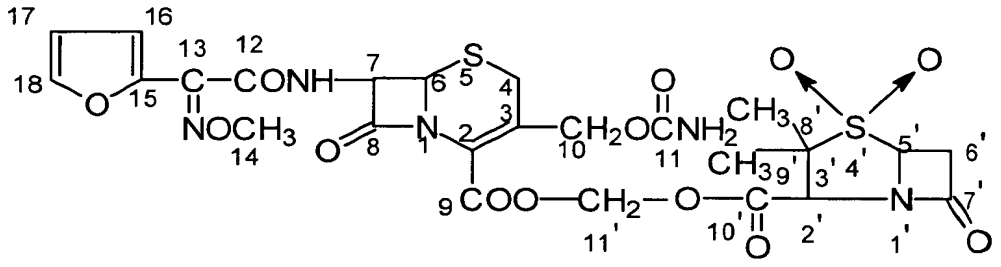
chemical shift ~~(υ)~~	genre
7.7107 (d,2H,J=8Hz)	C_{18}—H C_{20}—H
7.2326 (d,2H,J=8Hz)	C_{17}—H C_{19}—H
7.1304 (s,1H)	C_{15}—H
5.9820 (s,1H)	$C_{11'}$—H
5.7992 (d,1H,J=5Hz)	C_7—H
5.1806 (d,1H,J=5Hz)	C_6—H
4.9075 (m,1H)	$C_{5'}$—H
4.4946 (s,1H)	$C_{2'}$—H
4.0783 (s,3H)	C_{13}—H
3.9187 (m, 1H)	$C_{6'}$—H
3.5824 (m, 1H)	$C_{6'}$—H

3.6568,3.4267（ABq,2H,J=18Hz）	C_4－H
2.3703（s,3H ）	C_{21}－H
2.1841（s,3H）	C_{10}－H
1.5688（s,3H）	$C_{8'}$或or $C_{9'}$－H
1.4591（s,3H）	$C_{8'}$或or $C_{9'}$－H

Example 5:

The sodium salt of compound (II-2) 9.1g (0.025mol) was suspended in 100ml of ~~acetdimethylamide~~dimethyl acetamide, stirred and added 0.5g of 18 crownether-6, cooled the mixture to -15℃, added 9.4g (0.025mol) of the compound (III) (X=I), and then stirred 3hours. After the reaction finished, added 200ml of acetic ether and 200ml of water into the reaction solution, stirred thoroughly for 1 minutes and standed still to delaminate, seperated out acetic ether layer, and extracted water layer using 200ml of acetic ether, combined organic phase, sequentially washed with 150ml of diluted $NaHCO_3$ aqueous solution, 150ml of water and 100ml of saturated sodium chloride solution, and then decolored with activated carbon and dehydrated with magnesium sulfate anhydrous. Oily substances were acquired after decompressing and evaporating acetic ether, stirred these oily substances in 200ml of isopropanol for 30 minutes, filtrated, washed with small quantity of isopropanol, dried and got 12.5g of white solid of the compound YR-2, 85% yield. High Pressure Liquid Chromatography showed that the purity was 97.8%.

The compound YR-2 was confirmed by IR and ^1H NMR



IR (KBr disc)

absorption peak cm^{-1}	intensity	group
3485.34, 3376.65	Broad m	—C(=O)—NH_2

1790.33	Broad s	β-lactam
1737.4	s	-COOR
1683.66	s	-CONH-
1599.48	m	-C=C-
1537.01	m	-C=N-
1324.65, 1120.67	s	-C-O-C-

^{1}H NMR (DMSO d_6)

chemical shift ~~(υ)~~	genre
9.8037 (d,1H,J=8Hz)	CONH
7.8390 (broad s,1H)	C_{18}—H
6.6938 (d,1H,J=3Hz)	C_{16}—H
6.6364 (broad s,1H)	C_{17}—H
6.5—6.8 (broad s,2H)	—OC-NH_2
6.0299, 5.9129 (ABq,2H,J=6Hz)	$C_{11'}$—H
5.8576 (dd,1H,J=5Hz,8 Hz)	C_7—H
5.2520 (1H,J=5Hz)	C_6—H
5.1829 (m,1H)	$C_{5'}$—H
4.8770, 4.6316 (ABq,2H,J=13Hz)	C_{10}—H
4.5329 (s,1H)	$C_{2'}$—H
3.8912 (s,3H)	C_{14}—H
3.6821 (m,2H)	$C_{6'}$—H
3.5571, 3.2685 (ABq,2H,J=18Hz)	C_4—H
1.4874 (s,3H)	$C_{8'}$~~或~~ or $C_{9'}$ methyl H
1.3843 (s,3H)	$C_{8'}$~~或~~ or $C_{9'}$ methyl H

Example 6:

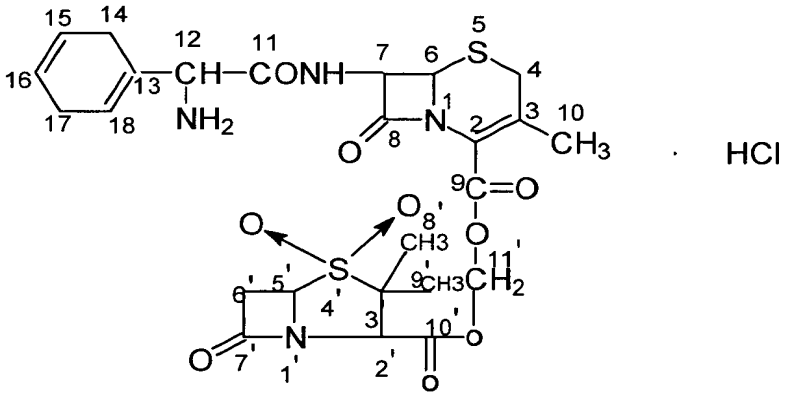
Added 5.6g (0.03mol) of tributylamine into 200ml of ~~acetdimethylamide~~dimethyl acetamide, stirred evenly and added 8.6g (0.025mol) of the compound (II-2), controlled at 20℃ and stirred to dissolve thoroughly, cooled to -15℃, added 9.4g (0.025mol) of the compound (III) (X=I), and stirred at -15℃ for 2 hours. Then, manipulated the rest steps according to the protocols used in example 5, and got 12.1g of YR-2, 82% yield. High Pressure Liquid

Chromatography showed that the purity was 98.2%. The analytic results of IR and ^{1}H NMR of the product were identical with those of example 5.

Example 7:

Added 3.6g (0.01mol) of the compound (II-3) into 36.5ml of ~~acetdimethylamide~~dimethyl acetamide, stirred and cooled to -10℃, added dropwisely 1.53g (0.01mol) of DBU to form solution, added 3.25 (0.01mol) of the compound (III) (X=Br), stirred and reacted for 12hours, added 100ml of acetic ether, 30ml of 3% $NaHCO_3$ solution and 50ml of 15% NaCl aqueous solution into the reaction solution, stirred for 10 minutes and standed still, separated out organic layer, washed with 50ml of 15% NaCl aqueous solution twice, decolored with activated carbon and dehydrated with magnesium sulfate anhydrous. Cooled to 0℃ and influxed with dry HCl gas to adjust pH to 2.5. At this momenmt, lots of solids were separated out, filtrated and washed with acetic ether three times, dried in vacuo and got 1.05g of hydrochloride of the compound YR-3. High Pressure Liquid Chromatography showed that the purity was 97%.

The hydrochloride of the compound YR-3 was confirmed by IR and ^{1}H NMR.



IR (KBr disc)

absorption peak (cm^{-1})	intensity	group
3450, 3250, 2900	Broad m	$-NH_2$, -NH
1784.3	Broad s	β-lactam and ester overlap
1697.12	m	-CONH-
1321.81, 1156.16	s	-C-O-C-

^{1}H NMR (DMSOd6)

chemical shift (~~υ~~)	genre
9.4450（d,1H,J=8Hz）	-CONH
8.4907（broad s,3H）	$-NH_3^+$
6.0133,5.9093（ABq,2H,J=6Hz）	$C_{11'}$—H
5.9599（broad s,1H）	C_{14}—H
5.7245（dd,1H,J=8 Hz）	C_7—H
5.6799（m,2H）	C_{16}—H, C_{17}—H
5.1979（dd,1H,J=4.6 Hz,1.6 Hz）	$C_{5'}$—H
5.1418（d,1H,J=4.6Hz）	C_6—H
4.5294（s,1H）	$C_{2'}$—H
4.3972（broad s,1H）	C_{12}—H
3.7418,3.6256（m,2H）	$C_{6'}$—H
3.4201,3.3014（ABq,2H,J=16Hz）	C_4—H
2.7197,2.5033（m,4H）	C_{15}—H ,C_{18}—H
2.0550（s,3H）	C_{10}—H
1.4807（s,3H）	$C_{8'}$—H ~~或~~ or $C_{9'}$—H
1.3738（s,3H）	$C_{8'}$—H ~~或~~ or $C_{9'}$—H

Example 8:

Added 3.6g (0.01mol) of the compound (II-3) into 36.5ml of ~~acetdimethylamide~~dimethyl acetamide, stirred and cooled to -10℃, added dropwisely 1.53g (0.01mol) of DBU to form clarifying solution, added 3.36 (0.009mol) of the compound (III) (X=I), stirred and reacted for 12hours, added 100ml of acetic ether and 150ml of pH1 HCl solution, stirred and delaminated, added 100ml of acetic ether into water layer, adjusted pH to 7.5 using saturated $NaHCO_3$ solution, delaminated, washed organic layer with 50ml mixture of 3% $NaHCO_3$ and 15% NaCl three times, decolored organic layer with activated carbon and dehydrated with magnesium sulfate anhydrous. Filtrated, cooled to 0℃ and influxed with dry HCl gas to adjust pH to 2.5. At this momenmt, lots of solids were separated out, filtrated, and washed the solids with acetic ether three times, dried in vacuo and got 0.9g of hydrochloride of the compound YR-3. High Pressure Liquid Chromatography showed that the purity was 94.5%. The analytic results of IR and ^1H NMR of the product were identical with those of example 7.

Example 9:

Added 3.6g (0.01mol) of the compound (II-3) into 36.5ml of ~~acetdimethylamide~~dimethyl acetamide, stirred and cooled to -10℃, added dropwisely 1.53g (0.01mol) of DBU to form clarifying solution, added 5.6g (0.015mol) of the compound (III) (X=I), stirred and reacted for 12hours, added 100ml of acetic ether and 150ml of pH1 HCl solution, stirred and delaminated, added 100ml of acetic ether into water layer, adjusted pH to 7.5 using saturated $NaHCO_3$ solution, delaminated, washed organic layer with 50ml mixture of 3% $NaHCO_3$ solution and 15% NaCl three times, decolored organic layer with activated carbon and dehydrated with magnesium sulfate anhydrous. Filtrated, cooled to 0℃, and influxed with dry HCl gas to adjust pH to 2.5. At this momenmt, lots of solids were separated out, washed the solids with acetic ether three times, dried in vacuo and got 0.95g of hydrochloride of the compound YR-3. High Pressure Liquid Chromatography showed that the purity was 95.5%. The analytic results of IR and ^1H NMR of the product were identical with those of example7.

Example10:

Manipulated according to example 7, substituted DBU with 0.01mol of dicyclohexyl amine, substituted bromomethyl ester with 0.01mol of the compound (III) (X=I), the reaction time was 1.5hours, got 1.15g of hydrochloride of YR-3. High Pressure Liquid Chromatography showed that the purity was 96%. The analytic results of IR and ^1H NMR of the product were identical with those of example 7.

Example 11:

Added 3.72g (0.01mol) of sodium salt of the compound (II-3) into 40ml of ~~acetdimethylamide~~dimethyl acetamide, stirred and cooled to 0℃, added 1.062g (0.01mol) of benzaldehyde and reacted for 10 hours at 0℃, cooled the reaction solution to -20℃, added 3.73g (0.01mol) of the compound (III) (X=I), stirred and reacted for 3 hours, added 110ml of dichloromethane, 50ml of 3% $NaHCO_3$ solution and 50ml of 15% NaCl aqueous solution, stirred for 10 minutes and standed still to delaminate, seperated out organic phase, washed with 100ml of pH7.5 phosphate buffer twice and with 100ml of saturated NaCl aqueous solution twice, decolored organic phase with activated carbon and dehydrated with magnesium sulfate anhydrous. Concentrated in vacuo and got oily substances, then added 50ml of aether and stirred to form 6.12g white crystal of the compound IV ~~(R_1=  R_2= $-CH_3$)~~ ,

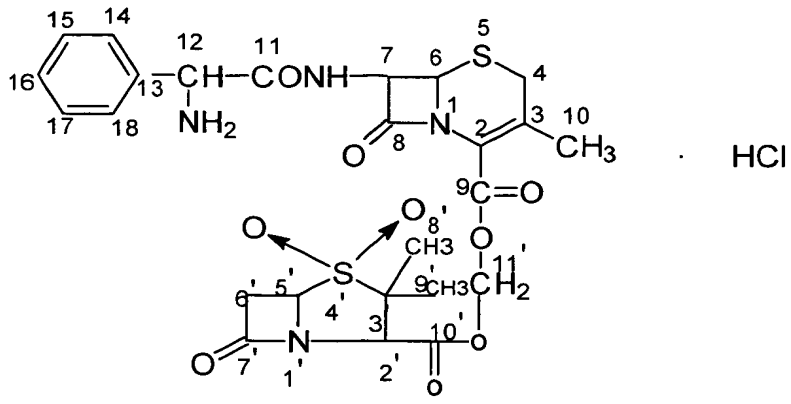
(R_1=$-CH_3$; R_2= ).

Dissolved 2.1g of *p*-toluenesulfonic acid and 2.1g of Grirnard reagent in 10ml of methanol, added 4.78g (0.01mol) of the above product into this solution at room temperature, stirred for 30 minutes, decompressed and evaporated methanol, added 30ml of dichloromethane and 30ml of pH 7.5 phosphate buffer into the residues, stirred for 10 minutes and standed still to delaminate, separated water layer, steamed with 25ml of dichloromethane three times. Washed the combined organic layer with saturated NaCl aqueous solution twice, removed the water layer, dried the organic phase with sodium sulphate anhydrous, cooled to 0℃ and influxed with dry HCl gas to adjust pH to 2.0, filtrated and collected solids, washed with small quantity of dichloromethane three times, dried in vacuo and got 3.8g of hydrochloride of YR-3. High Pressure Liquid Chromatography showed that the purity was 96.5%. The analytic results of IR and ^{1}H NMR of the product were identical with those of example 7.

Example12:

Added 3.65g (0.01mol) of the compound (II-4) into 42ml of ~~acetdimethylamide~~dimethyl acetamide, stirred and cooled to -15℃, added dropwisely 1.53g (0.01mol) of DBU, stirred for 30 minutes, added 3.25g (0.01mol) of the compound (III) (X=Br) at the same temperature, stirred and reacted for13hours, added 100ml of dichloromethane and 100ml of pH7.5 phosphate buffer, stirred for 10 minutes and standed still to delaminate, sequentially washed the organic phase with 50ml of pH7.5 phosphate buffer twice and saturated NaCl aqueous solution twice, then decolored with activated carbon, and dehydrated with magnesium sulfate anhydrous. Cooled to 0℃ and influxed with dry HCl gas to adjust pH to 2.0, filtrated and collected solids, washed with dichloromethane three times, dried in vacuo and got 1.8g of hydrochloride of YR-4. High Pressure Liquid Chromatography showed that the purity was 97.2 %.

The structure of hydrochloride of the compound YR-4 was confirmed by IR and ^{1}H NMR.



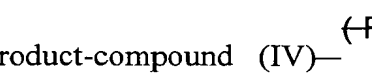
IR (KBr disc)

absorption peak（cm^{-1}）	intensity	group
3450, 3250, 2930.55	Broad m	$-NH_2$, -NH
1784.63	Broad s	β-lactam and ester overlap
1697.07	m	-CONH-
1321.36，1156.95	s	-C-O-C-

^1H NMR (DMSOd6)

chemical shift ~~(υ)~~	genre
9.5752（d,1H,J=8Hz）	-CONH-
8.8117（s,3H）	NH_3^+
7.5479—7.4162（m,5H）	$C_{14,\ 15,\ 16,\ 17,\ 18}$—H
6.0003，5.8978（ABq,2H,J=6Hz）	$C_{11'}$—H
5.7593（dd,1H,J=8 Hz）	C_7—H
5.1956（dd,1H,J=4.5 Hz,1.5 Hz）	$C_{5'}$—H
5.0472（s,1H）	C_{12}—H
5.0394（d,1H,J=4.7Hz）	C_6—H
4.5245（s,1H）	$C_{2'}$—H
3.7049（dd,1H,J=15 Hz,4.5Hz）	$C_{6'}$—H
3.3689（dd,1H,J=15 Hz,1.5Hz）	$C_{6'}$—H
3.5419，3.2743（ABq,2H,J=18Hz）	C_4—H
2.0154（s,3H）	C_{10}—H
1.4477（s,3H）	$C_{8'}$~~或~~or $C_{9'}$—H
1.3674（s,3H）	$C_{8'}$~~或~~or $C_{9'}$—H

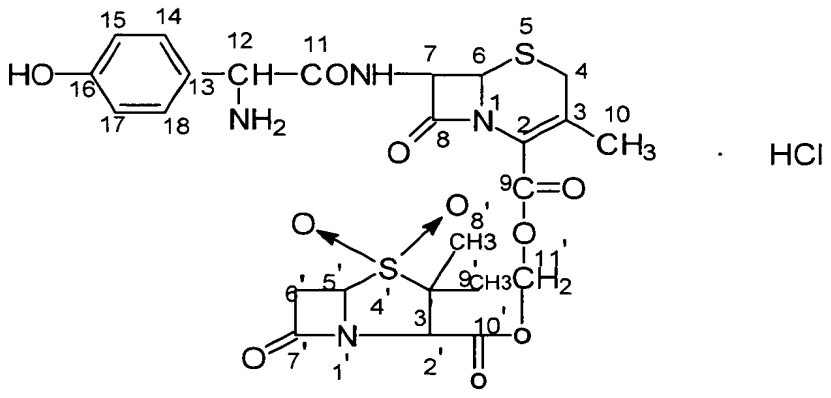
Example 13:

Added 4.03g (0.01mol) of potassium salt of the compound (II-4) into 50ml of ~~acetdimethylamide~~dimethyl acetamide, stirred and cooled to 0℃, added 1.062g (0.01mol) of benzaldehyde and reacted for 8hours at 0℃~5℃, cooled the reaction solution to -15℃, added 3.73g (0.01mol) of the compound (III) (X=I), stirred and reacted for 2 hours. The other reaction steps were performed according to example 9, got 6.05g of white crystal of intermediate product-compound (IV)—  (R_1=-CH_3 ; R_2= ), and finally got 3.65g of hydrochloride of YR-4. The analytic results of IR and ^1H NMR of the product were identical with those of example 10.

Example14:

3.65g (0.01mol) of the compound (II-5) was suspended in 37ml of dimethylformamide, cooled it to -20℃, added 1.51g (0.01mol) of DBU and stirred to dissolve, added 3.73 (0.01mol) of the compound (III) (X=I), stirred for 30minutes, added 37ml of acetic ether and 80ml of aqueous solution consisting of 15% NaCl and 5% $NaHCO_3$, stirred for 10 minutes and delaminated, separated organic layer and washed with the above aqueous solution consisting of 15% NaCl and 5% $NaHCO_3$ twice, dehydrated with magnesium sulfate anhydrous, filtrated and influxed with dry HCl gas to adjust pH to 2-3. After crystal was separated out, continued stirring for 10 minutes, filtrated and washed with small quantity of acetic ether, dried in vacuo and got 3.7g white crystal of hydrochloride of the compound YR-5. High Pressure Liquid Chromatography showed that the purity was 95.6%.

The hydrochloride of the compound YR-5 was confirmed by IR and ^1H NMR.



IR (KBr disc)

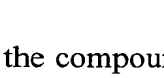
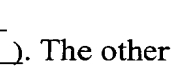
absorption peak (cm^{-1})	intensity	group
3400, 3200, 2900	Broad m	$-NH_2$, -NH, -OH
1779.61	Broad s	β-lactam and ester overlap
1693.71	m	-CONH-
1320.64, 1183.04	s	-C-O-C-

^1H NMR (DMSOd6)

chemical shift (~~υ~~)	genre
9.1868 (s,1H)	-OH
9.4460 (d,1H,J=8Hz)	-CONH-
8.6479 (s,3H)	NH_3^+
7.2958 (d,2H,J=8.5Hz)	C_{14},C_{18}—H
6.7917 (d,2H,J=8.5Hz)	C_{15},C_{17}—H
5.9990, 5.8974 (ABq,2H,J=6Hz)	$C_{11'}$—H
5.7392 (dd,1H,J=8 Hz,4.5Hz)	C_7—H
5.1907 (d,1H,J=4.0Hz)	$C_{5'}$—H
5.0478 (d,1H,J=4.5Hz)	C_6—H
4.9200 (broad s ,1H)	C_{12}—H
4.5206 (s,1H)	$C_{2'}$—H
3.6997 (dd,1H,J=16.5Hz,4.0Hz)	$C_{6'}$—H
3.3881 (dd,1H,J=16.5Hz,)	$C_{6'}$—H
3.5535, 3.2734 (ABq,2H,J=18Hz)	C_4—H
2.0171 (s,3H)	C_{10}—H

1.4759（s,3H）	C_8-~~或~~or C_9-—H
1.3674（s,3H）	C_8-~~或~~or C_9-—H

Example 15:

Added 4.01g (0.01mol) of potassium salt of the compound (II-5) into 15ml of dimethylformamide, cooled to 0℃, added 1.27g (0.012mol) of benzaldehyde and stirred and reacted for 8 hours, added 3.73g (0.01mol) of the compound (III) (X=I), stirred and reacted for 30 minutes, added 40ml of acetic ether and 80ml aqueous solution consisting of 15% NaCl and 5% $NaHCO_3$, stirred for 10 minutes and delaminated, separated out organic layer and washed with saturated NaCl aqueous solution, dehydrated with magnesium sulfate anhydrous, filtrated, decompressed and evaporated organic solvent, and then added 50ml of isopropyl ether and stirred to form 6.1g of yellow crystal of the compound (IV)~~(R_1= HO—  R_2=—CH_3)~~ (<u>R_1=-CH_3; R_2=</u>  <u>)</u>. The other reaction steps were performed according to example 9, got 2.56g of hydrochloride of YR-5. High Pressure Liquid Chromatography showed that the purity was 97.2%. The analytic results of IR and ^1H NMR of the product were identical with those of example 12.

In order to further demonstrate the antibacterial effects of the compounds according to the invention and use thereof, YR-1 and YR-2 was ~~chosed~~ <u>chosen</u>to perform in vitro antibacterial activity experiment, ex vivo antibacterial activity experiment after mouse is administered and mouse maximum tolerable dose experiment, all of which were accomplished by Shanghai Institute of Pharmaceutical Industry.

Effectiveness example 1: in vitro antibacterial activity experiment

1. Experiment materials: tested samples YR-1, YR-2 were the ones prepared in example 1 and example 5 respectively, control samples were cefetamet sodium (CTM), cefuroxime sodium (CRX), cefetamet sodium + sulbactam sodium (CTM + SBT) (molar ratio 1:1), cefuroxime + sulbactam sodium (CRX + SBT) (molar ratio 1:1). All control samples (have been sold on the market) were supplied by Zhejiang Yongning pharmaceutical factory.

2. Experiment bacteria strains:

Staphylococcus aureus 26003, Diplococcus lanceolatus 31002, *E coli* 44102, Shigella sonnet 51081, Shigella bogdii 51313, Shigella flexneri 51573, Proteus mirabilis 49005, Bacillus proteus 49085, Proteus morganii 49086, Pseudomonas aeruginosa 10124, Bacillus pneumoniae 46101, Salmonella enteritidis 50041, Salmonella typhi 50097, Citrobacter 48017, Candida ciferii 41002 were supplied by Shanghai Hygienic and Antiepidemic Station.

Staphylococcus epidermidis 26069 and Bacillus aerogenes 45102 were supplied by Beijing Drug & Biology Product Appraisal Bureau.

Diplococcus lanceolatus 0031 was supplied by Shanghai First People's Hospital.

3. Culture medium:

Mueller-Hinton Agar (M.H) culture medium, batch number 20040528 (Shanghai Reagent Supply Research Center, China Diarrhea Disease Control)_~~o~~_.

4. Experiment methods:

Adopted agar double dilution, inoculated using multipoint inoculator, inoculated 10^5CFU/ML each point, cultured for 24 hours at 37℃, observed and recorded the results, set the least concentration of the antibacterial drug that can inhibit bacteria growth as the minimum inhibitory concentration (MIC).

5. Experiment results:

MIC of the drug to bacteria (μg/ml)

Bacteria strain	cefetamet (CTM)	cefuroxime (CRX)	Cefetamet + Sulbactam (CTM + SBT)	Cefuroxime + Sulbactam (CRX + SBT)	YR-1	YR-2
Staphylococcus aureus 26003	100	1.56	50	3.13	100	6.25
Diplococcus lanceolatus 31002	>100	25	50	25	100	25

E. coil 44102	0.78	6.25	0.78	6.25	1.56	12.5
Shigella sonnet 51081	0.39	0.78	0.195	1.56	0.39	1.56
Shigella bogdii 51313	0.39	1.56	0.195	1.56	0.39	1.56
Proteus mirabilis 49005	0.195	1.56	0.39	3.13	0.098	3.13
Bacillus proteus 49085	0.195	0.78	0.39	1.56	0.195	0.78
Proteus morganii 49086	>100	100	3.13	12.5	0.78	25
Pseudomonas aeruginosa 10124	100	100	100	100	100	100
Bacillus pneumoniae 46101	0.78	25	0.78	12.5	0.78	12.5
Salmonella enteritidis 50041	0.78	6.25	0.78	6.25	1.56	12.5
Salmonella typhi 50097	0.78	3.13	0.78	3.13	1.56	6.25
Citrobacter 48017	1.56	1.56	1.56	3.13	1.56	3.13
Bacillus aerogenes 45102	0.195	3.13	0.39	6.25	0.39	6.25
Candida ciferii 41002	0.78	25	0.78	25	0.78	50
Shigella flexneri 51573	0.78	1.56	0.78	3.13	0.39	3.13
Staphylococcus epidermidis 26069	12.5	0.39	25	0.78	50	0.78
Diplococcus lanceolatus 0031	25	0.78	25	0.78	50	0.78

6. Conclusions:

Both YR-1 and YR-2 have antibacterial activity in vitro. Their antibacterial activities are nearly equal to CTM + SBT and CRX + SBT respectively. Both YR-1 and YR-2 have stronger antibacterial activities to β-lactamase releasing Gram negative bacteria than those of CTM or CRX used solely, for example, to Proteus morganii 49086, MICs of CTM and CRX are >100mg/ml and 100mg/ml respectively, while MICs of YR-1 and YR-2 are 0.78mg/ml and 25mg/ml respectively, the antibacterial activities enhanced one hundred times and four times respectively. CTM belongs to the third generation of cephalosporin, which has no effects to Gram positive bacteria and Pseudomonas aeruginosa, CRX belongs to the second generation of cephalosporin, which has weak effects to Gram positive bacteria and has no effects to

Pseudomonas aeruginosa, and YR-1 and YR-2 show the same results. To some bacteria without enzyme releasing, YR-1 and YR-2 show the same antibacterial activities as CTM and CRX.

Effectiveness example 2: ex vivo antibacterial activity experiment after mouse is administered.

1. Experiment materials: the sources of tested samples (YR-1, YR-2) and control samples (CTM, CRX, CTM+SBT 1:1 and CRX+SBT 1:1) are the same as above.

2. Experiment bacteria strains:

Bacillus proteus 49085, Proteus morganii 49086, inoculated 10^5CFU/ML each dish.

3. Culture medium:

Mueller-Hinton Agar (M.H) culture medium, batch number 000707 (Shanghai Reagent Supply Research Center, China Diarrhea Disease Control)。

4. Experiment animals:

Strain: Kunming mice; source: Animal Facility of Shanghai Institute of Pharmaceutical Industry; certificate number: Hudonghezhengzi No.107; animal numbers: 120; Sex: same number of male and female mice; Body weight: 18~21g; fast time: 16 hours.

5. Experiment methods:

Group 1: CTM, CRX, CTM+SBT 1:1, CRX+SBT 1:1 were given at a dosage of 500mg/kg intravenously, collecting blood at 10minutes, 30minutes, 1hour, 2hours, 4hours, 8hours after administration.

Group 2: YR-1, YR-2 were given at a dosage of 1000mg/kg via intragastric administration, collecting blood at 10minutes, 30minutes, 1hour, 2hours, 4hours, 8hours after administration.

Kunming mice were randomly divided into several groups according to body weight of empty stomach and sex. Collecting blood of three mice at different time point, anticoagulated with heparin, centrifugated and separated plasma, quantitative spotting, semi-quantitated antibacterial activity according to the size of bacterial inhibition ring.

6. Experiment results:

Results of ex vivo antibacterial activity to Bacillus proteus 49085

sample		Cefetamet sodium (CTM)	Cefuroxime sodium (CRX)	Cefetamet sodium+ sulbactam (CTM+ SBT)	cefuroxime + sulbactam (CRS+ SBT)	YR-1	YR-2
No.		1	2	3	4	6	5
administration manner		intravenous injection	intravenous injection	intravenous injection	intravenous injection	intragastric administration	intragastric administration
Dosage		500mg/kg	500mg/kg	500mg/kg	500mg/kg	1000mg/kg	1000mg/kg
collecting blood time point and antibact-erial activity	10min	+++	+++	++	++	—	—
	30min	+++	+++	++	++	+	+
	1hr	+++	++	+	+	+	+
	2hr	++	+	+	+	+	+
	4hr	±	+	+	+	+	+
	8hr	—	—	—	—	+	±

Results of ex vivo antibacterial activity to Proteus morganii 49086

sample		Cefetamet Sodium (CTM)	Cefuroxime sodium (CRX)	Cefetamet sodium+ sulbactam (CTM+ SBT)	cefuroxime + sulbactam (CRS+ SBT)	YR-1	YR-2
No.		1	2	3	4	6	5
administration manner		intravenous injection	intravenous injection	intravenous injection	intravenous injection	intragastric administration	intragastric administration
dosage		500mg/kg	500mg/kg	500mg/kg	500mg/kg	1000mg/kg	1000mg/kg
collecting blood time point and antibacte-rial activity	10min	++	+	++	+	—	—
	30min	+	+	++	+	+	+
	1hr	+	+	+	+	+	+
	2hr	+	+	+	+	+	+
	4hr	±	+	±	±	+	+
	8hr	—	—	—	—	+	+

7. Conclusions:

Antibacterial activities can be detected in the blood of mice orally adminstered with YR-1 and YR-2. Mice show stable and persistent blood drug concentrations after given YR-1 and YR-2 via intragastric administration. Antibacterial activities of YR-1 and YR-2 can be detected even 8 hours after administration, while considering that CTM, CRX, CTM+SBT and CRX+SBT can not be absorbed via oral administration, they all are given via intravenous injection, and

they are easy to reach blood peak concentration and show stronger antibacterial activities, but their metabolism is much more quicker. Antibacterial activities of CTM, CRX, CTM+SBT and CRX+SBT can not be detected 8 hours after administration. It demonstrates that YR-1 and YR-2 have longer half-life period and prolonged effects.

Effectiveness example 3: mouse maximum tolerable dose experiment

1. Experiment materials: tested samples (YR－1,YR－2) are the same as above.

2. Experimental animals:

Strain: Kunming mice; source: Animal Facility of Shanghai Institute of Pharmaceutical Industry; certificate number: Hudonghezhengzi No.107; animal numbers: 20; Sex: same number of male and female mice; Body weight: 18~21 g; fast time: 16 hours.

3.Dosage:

Preparation of samples: 5g/kg (prepared with 5% carboxymethyl cellulose CMC); volume accepted: 0.6ml/20g body weight/each time; administration times: once; dosage: 5g/kg/24hr

4. Administration manner: intragastric administration

5. Experiment methods:

20 mice, 10 male and 10 female, were given YR-1 or YR-2 via intragastric administration, observed manifestation of mice toxic symptom immediately after administration, recorded mice death number.

6. Observing index:

Observed manifestation of mice toxic symptom immediately after administration, observed twice each day (morning and evening).

Death: recorded mice death number during observation, autopsied dead mice immediately and observed changes of main organs of mice including heart, liver, spleen, lung, kidney etc with naked eyes; if abnormality was observed with naked eyes, performing pathologic examination.

Toxic reaction: recorded behaviors of mice, skin, respiration, urination and defecation, appetite, checked if there are abnormal secretions appeared in the nose, eye and mouth.

Observation period: 7 days, killed all the surviving mice after observation period, and autopsied to see if there is any abnormality existed in the mice organs.

7. Experiment results:

After fasted for 16 hours and given YR-1 or YR-2, mice showed no obvious abnormal symptoms of toxic reactions, mice activities had no obvious changes, and no abnormalities were observed in the organs after killed.

Acute toxicity test of YR-1 and YR-2 on mice

sample	group	Dosage (g/kg/24hr)	Mice number	Death number during observation							Death rate %
				1	2	3	4	5	6	7 (day)	
YR-1	female	5	10	0	0	0	0	0	0	0	0
	male	5	10	0	0	0	0	0	0	0	0
YR-2	female	5	10	0	0	0	0	0	0	0	0
	male	5	10	0	0	0	0	0	0	0	0

8. Conclusions:

Due to low toxicity of YR-1 and YR-2, there was no way to determine LD_{50}, maximum tolerable dose test was performed, we can learn from the results that LD_{50}>5g/kg. This demonstrates that YR-1 and YR-2 are kinds of safe and low toxical drugs that could be taken orally.